KASP genotyping explained

The purpose of this document is to provide an explanation of how KASP™ genotyping chemistry works, and also to provide information on how data is collected and analysed in our service laboratory.

KASP overview

KASP genotyping assays are based on competitive allele-specific PCR and enable bi-allelic scoring of single nucleotide polymorphisms (SNPs) and insertions and deletions (indels) at specific loci. The SNP-specific KASP Assay Mix and the universal KASP-TF Master Mix are added to DNA samples, a thermal cycling reaction is then performed, followed by an end-point fluorescent read. Bi-allelic discrimination is achieved through the competitive binding of two allele-specific forward primers, each with a unique tail sequence that corresponds with two universal FRET (fluorescence resonant energy transfer) cassettes; one labelled with FAM™ dye and the other with HEX™ dye.

Detailed explanation of KASP genotyping chemistry

KASP genotyping assays are based on competitive allele-specific PCR and enable bi-allelic scoring of single nucleotide polymorphisms (SNPs) and insertions and deletions (indels) at specific loci.

The SNP-specific KASP Assay Mix and the universal KASP-TF Master Mix are added to DNA samples, a thermal cycling reaction is then performed, followed by an end-point fluorescent read. The KASP Assay Mix contains three assay-specific non-labelled oligos: two allele-specific forward primers and one common reverse primer.

The allele-specific primers each harbour a unique tail sequence that corresponds with a universal FRET (fluorescence resonant energy transfer) cassette; one labelled with FAM™ dye and the other with HEX™ dye. The KASP-TF Master Mix contains the universal FRET cassettes, ROX™ passive reference dye, Taq polymerase, free nucleotides and $MgCl_2$ in an optimised buffer solution.

During thermal cycling, the relevant allele-specific primer binds to the template and elongates, thus attaching the tail sequence to the newly synthesised strand. The complement of the allele-specific tail sequence is then generated during subsequent rounds of PCR, enabling the FRET cassette to bind to the DNA. The FRET cassette is no longer quenched and emits fluorescence. Bi-allelic discrimination is achieved through the competitive binding of the two allele-specific forward primers. If the genotype at a given SNP is homozygous, only one of the two possible fluorescent signals will be generated. If the genotype is heterozygous, a mixed fluorescent signal will be generated.

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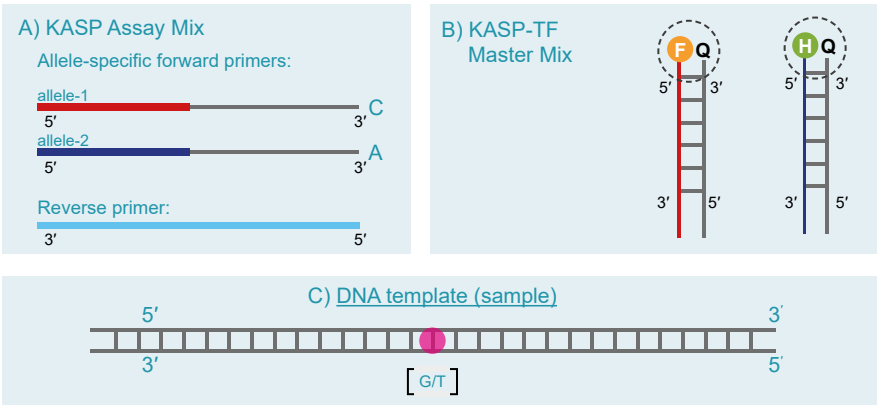
How KASP works

1. Assay components:

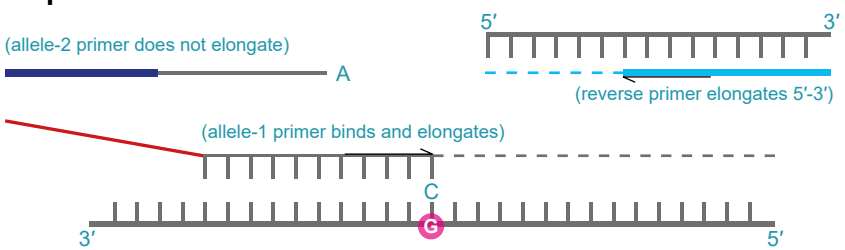
A) KASP Assay Mix: consists of 2 allele specific primers and 1 reverse primer.

B) KASP-TF Master Mix: contains universal fluorescent probes, Taq polymerase and dNTPs in an optimised buffer solution.

C) Sample DNA: DNA contains the SNP of interest.



2. Denatured template and annealing components – PCR round 1:

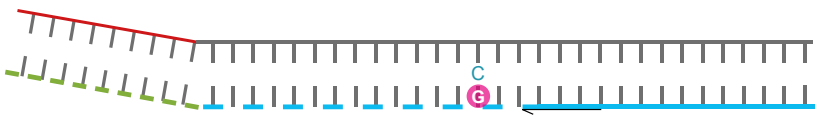


In the first round of PCR, one of the allele-specific primers matches the target SNP and with the common reverse primer, amplifies the target region.

Legend

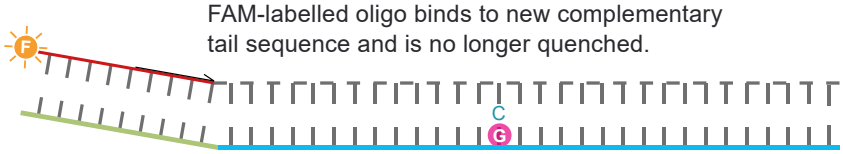
- Allele-1 tail FAM-labelled oligo sequence
- Allele-2 tail HEX-labelled oligo sequence
- Common reverse primer
- F — FAM dye
- H — HEX dye
- Target SNP
- Q — Quencher

3. Complement of allele-specific tail sequence generated – PCR round 2:

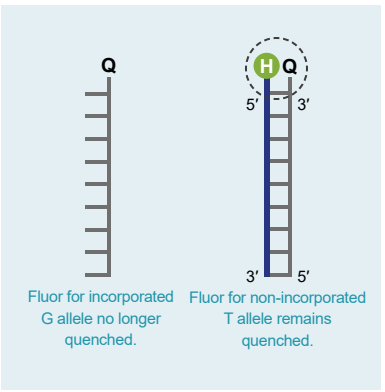


Reverse primer binds, elongates and makes a complementary copy of the allele-1 tail.

4. Signal generation – PCR round 3:



In further rounds of PCR, levels of allele-specific tail increase. The fluor labelled part of the FRET cassette is complementary to new tail sequences and binds, releasing the fluor from the quencher to generate a fluorescent signal.



Analysis of genotyping data in our service laboratory

All assays for human samples are tested on LGC Biosearch Technologies' in-house validation DNA prior to being run on customer samples. Assays are deemed to be working successfully if clusters are distinct and call rates are consistently high. If samples are non-human, the assay is validated on DNA supplied by the customer.

The data is automatically quality control checked on a per SNP basis. No template controls (NTCs) are included on each plate to enable the detection of contamination or non-specific amplification – these samples must not amplify during the reaction. The number of genotypes that are callable must be greater than 90% and minor allele frequency should be greater than 2% unless the SNP is known to be a very low frequency. A chi-squared value (X2) is also generated that assesses distribution in multinomial datasets based on the Hardy-Weinberg equation.

Following completion of the initial 35 cycles of PCR, all genotyping reaction plates run at Biosearch Technologies are read on a BMG PHERAStar plate reader. This initial read data is visually inspected by a member of the genotyping team to assess the progression of the PCR reaction. The plates are then recycled (3 cycles per recycle step) and read after each recycle step. The laboratory operator visually inspects the read data after each recycle step and, once they are satisfied that the PCR reaction has reached endpoint, identifies plates as completed. At this stage, our in-house Kraken software will automatically call genotypes for your samples. Your project manager will access the plate read data in Kraken and perform a detailed analysis of the data. This may require them to correct the automatically called genotypes that Kraken has given. Version one of your genotyping results is then exported within the Kraken system. A second project manager will then second check these results and verify or change them in collaboration with your project manager. The results are then ready to send to you, the customer.

For any queries about running KASP reactions in your laboratory, please contact the technical support team at techsupport@lgcgroup.com.

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